bio-protocol Preprint

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Lipid Extraction From Cultured Adipocytes

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An abbreviated version of this protocol was published in eLIFE in Apr 2023

Aggregating in vitro-grown adipocytes to produce macroscale cell-cultured fat tissue with tunable lipid compositions for food applications

DOI: 10.7554/eLife.82120

Detailed protocol

MTBE Extraction

Extraction performed in 2 ml snap cap microcentrifuge tubes, final sample collection in 1.5 ml tubes
Warning: Perform in fume hood.

Equipment Needed:
Microcentrifuge
Polypropylene tubes
Vaccum centrifuge/Nitrogen dryer

Required Solvents:
500 ul methanol per sample (can aliquot into polypropylene)
875 ul MTBE (methyl tert butyl ether) per sample (aliquot working volume into glass e.g scintillation vial when performing the experiment)
625 ul water per sample

Protocol:
1. Add 500 ul of methanol to cell pellet (or 5-10 mg of crush/ground/homogenized adipose tissue) and vortex 1 min
2. Add 625 ul of MTBE and incubate for 1h with occasional vortexing or with shaking/rocking
    MTBE drips out of 1 ml pipette tip so be careful
3. Add 625 ul of water and vortex for 1 min
4. Spin down samples at 10,000 x g for 5 min
5. Transfer the upper organic phase to a new polypropylene snap-cap tube (1.5 ml)
6. Add 250 ul of MTBE to reextract the remaining aqueous phase (vortex for 1 min then spin down solution)
    Aq and Organic phases should be mixed up after vortexing, they should not stay separate. Repeat vortexing otherwise
7. Transfer the upper organic phase to previously extracted MTBE/organic phase (in the snap cap tube)
8. Dry the double extracted MTBE phases in a vaccum centrifuge, or under nitrogen.

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Yuen Jr, J. S. and Kaplan, D. (2023). Lipid Extraction From Cultured Adipocytes. Bio-protocol Preprint. bio-protocol.org/prep2318.
2. Yuen Jr, J. S. K., Saad, M. K., Xiang, N., Barrick, B. M., DiCindio, H., Li, C., Zhang, S. W., Rittenberg, M., Lew, E. T., Zhang, K. L., Leung, G., Pietropinto, J. A. and Kaplan, D. L.(2023). Aggregating in vitro-grown adipocytes to produce macroscale cell-cultured fat tissue with tunable lipid compositions for food applications. eLIFE. DOI: 10.7554/eLife.82120

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